Blood Cells, Molecules and Diseases 75 (2019) 30–34

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

Association of fetal hemoglobin level with frequency of acute pain episodes in sickle cell disease (HbS-only phenotype) patients

Bimal Prasad Jit[a,b], Pradeep Kumar Mohanty[b,c], Prasanta Purohit[b,d], Kishalaya Das[b], Siris Patel[b], Satyabrata Meher[b], Jyoti Ranjan Mohanty[a], Shalini Sinha[a], Rajendra Kumar Behera[e], Padmalaya Das[a,*]

[a] School of Life Sciences, AIPH University, Bhubaneswar, Odisha, India
[b] Sickle Cell Clinic and Molecular Biology Laboratory, Veer Surendra Sai Institute of Medical Sciences and Research, Burla, Sambalpur, Odisha, India
[c] Department of Medicine, Veer Surendra Sai Institute of Medical Sciences and Research, Burla, Sambalpur, Odisha, India
[d] Multidisciplinary Research Unit, Maharaja Krishna Chandra Gajapati Medical College, Berhampur, Odisha, India
[e] School of Life Sciences, Sambalpur University, Jyoti Vihar, Burla, Sambalpur, Odisha, India

ARTICLE INFO

Keywords:
Sickle cell disease
Fetal hemoglobin
Alpha thalassemia
Acute pain episodes

ABSTRACT

Background: Sickle cell disease (SCD) is a Mendelian single gene disorder with highly variable phenotypic expression. In the present study, we analyzed the influence of HbF, alpha thalassemia and other hematological indices to determine their association with acute pain episodes.

Method: This case control study consisted of SCD subjects with HbS phenotype experiencing three or more acute pain episodes in last twelve months (cases) and without any episode of acute pain during last twelve months (controls). Hematological parameters, HbF, and presence of alpha thalassemia were assessed in all subjects.

Results: A statistically significant difference between HbF levels ($P < 0.025$, $\chi 2$ test) and alpha thalassemia ($P < 0.008$, $\chi 2$ test) was observed between controls and cases group. Univariate analysis indicated that increased HbF levels > 25% (OR: 0.37, 95% CI: 0.18-0.77, $P < 0.008$) and presence of alpha thalassemia (OR: 0.53, 95% CI: 0.33–0.85, $P < 0.009$) provided protection, while multivariate analysis revealed significant protection was attributable only by higher HbF levels (OR: 0.39, 95% CI: 0.17–0.88, $P < 0.025$). Significantly higher HbF levels were observed only in the 11–20 age group of cases in comparison to controls (Student's *t*-test, $P < 0.001$).

Conclusion: Higher concentrations of HbF are associated with protection against frequent episodes of acute pain crisis in SCD patients.

1. Introduction

Sickle cell disease (SCD), a monogenic disorder, is a major public health concern in several parts of India. The sickle gene is mainly concentrated in tribal and scheduled caste population of central India, where carrier frequencies range between 5 and 40% [1]. The disease is highly prevalent in the western parts of State of Odisha [1,2].

The most important complication associated with SCD is the vasoocclusive painful crisis (VOC). Although vasoocclusion is a complex phenomenon, HbS polymerization has been proposed as an essential mechanism for its occurrence [3]. Polymerization of HbS in sickle erythrocytes leads to increasing cell stiffness, significant increase in blood viscosity, and occasionally occlusion of the vasculature [4]. VOC frequently leads to acute pain, tissue infarction, long term organ damage, and significantly shortened life expectancy [5,6].

Phenotypic expression of SCD is highly variable, though it is a single gene mutation disease. The clinical severity of the disease varies from occasional symptoms to multiple episodes of acute painful crisis and severe anemia requiring blood transfusion [7]. Higher levels of fetal hemoglobin (HbF), certain beta globin gene cluster haplotype, association of alpha thalassemia, and *Xmn*I polymorphism have been considered to provide protection against severe complications [7], and higher mean corpuscular hemoglobin concentration (MCHC), leukocytosis and thrombocytosis [8,9] have been suggested to contribute to higher frequency of clinical complications. However, the single most important factor that provides protection against frequent episodes of painful crisis is reported to be higher HbF ($\alpha 2\gamma 2$) levels, which presumably inhibits HbS polymerization [10].

* Corresponding author.
E-mail address: pdas@aiph.ac.in (P. Das).

https://doi.org/10.1016/j.bcmd.2018.12.003
Received 6 September 2018; Received in revised form 19 December 2018; Accepted 19 December 2018
Available online 20 December 2018

Watson in 1948, proposed that lack of clinical symptoms in newborns with SCD may have been contributed by higher concentrations of HbF. She observed that the occurrence of clinical symptoms of SCD started with a decline in HbF levels [11]. Since then, HbF has considered as one of the important modifiers of clinical course of SCD [12]. Blood HbF concentrations are widely variable among SCD patients in various geographical regions [13], high in Arab-Indian (AI haplotype) [14] or Senegal haplotypes [15], intermediate in Benin haplotypes [16] and low in Bantu haplotypes [17]. Patients of SCD from the Middle East and India co-inherited AI haplotypes with additional genetic mutations that resulted in the persistence of HbF production, which is possibly responsible for less severe disease in these populations [18].

Higher HbF levels were found to lower the frequency of acute painful crisis, leg ulcers, osteonecrosis and acute chest syndromes [19–23]. The antisickling agent hydroxyurea (HU) decreases HbS polymerization by increasing HbF levels [24].

However, AI haplotype SCD patients with higher HbF levels are not fully protected from complications [25] and about 10 to 20% of adults did not show adequate response to HU therapy [26]. Yet others found little protective effect of higher HbF against sickle complications [27]. Some of these differences are presumably contributed by difference in HbF level in different haplotypes [10], methods of estimation of HbF (Alkali denaturation method vs HPLC) [28], and the type of study design.

Indian SCD patients have comparatively higher frequencies of alpha thalassemia, higher HbF levels, higher total hemoglobin and red cell counts, and lower mean cell volume, mean cell hemoglobin concentration, and reticulocyte counts. The clinical manifestation in Indian SCD patients with a high level of HbF is usually considered mild [29], but other studies have shown in spite of higher HbF levels and association of alpha thalassemia, a significant proportion of SCD patients in Odisha develop acute pain episodes [30]. The present study attempts to determine whether a subgroup of SCD patients with higher HbF levels are protected from frequent acute pain episodes using a case control study design. The cases were patients of SCD (HbS-only phenotype) with multiple acute pain episodes and the controls were SCD patients (HbS-only phenotype) without acute pain episodes in last 12 months.

2. Material and methods

2.1. Study design and the study site

This was a case control study undertaken at the Sickle Cell Centre and Molecular Biology Laboratory of Veer Surendra Sai Institute of Medical Sciences and Research (VIMSAR), Burla, Odisha located in eastern part of India. It is a tertiary care, referral hospital for about 12 million people residing in western Odisha and adjacent parts of Chhattisgarh state. A significant proportion of the population of these areas is tribal (aboriginal) and the prevalence of hemoglobin disorders is very high in these communities [2]. The patient recruitment was done from January 2016 to July 2018. The Sickle Cell Centre receives cases of sickle cell disease from multiple sources including the referred cases from districts with a positive screening test, clinically suspected cases from other departments of the VIMSAR, referred from other public and private health care facilities, family members of the known SCD cases, and those attending for voluntary screening.

2.2. Diagnosis of sickle cell disease

All new cases attending sickle cell centre from whichever source were screened for sickle cell disease using agar gel electrophoresis at pH at 8.6.Those found positive in the electrophoresis were subjected for confirmation and quantification of different hemoglobin fractions by high performance liquid chromatography (HPLC) using the VARIANT II Hemoglobin (Hb) testing system; (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's guidelines. HbF concentration was estimated from the HPLC. Complete blood count (CBC) was done using automated hematology analyzer (Sysmex KX-21; Sysmex Corporation, Kobe, Japan).

2.3. Selection of study subjects

HPLC confirmed, newly diagnosed SCD (HbS-only phenotype) cases were divided into two groups according to their frequency of acute pain episodes in last twelve months. Those having more than three acute pain episodes in last twelve months were considered as cases, and those without a single acute pain episode in last twelve months were considered as controls. An acute pain episode was defined as the occurrence of pain in the extremities, back, abdomen, chest, or head that lasted at least 2 h, led to a clinic visit and couldn't be explained except by sickle cell disease [12]. We excluded children 10 years of age or below for the present study as HbF levels stabilize at about 10 years age [26]; patients of other sickle cell syndromes such as HbS-β thalassemia, HbSE, HbSC, $HbSD^{-Punjab}$; had received blood transfusion in last 3 months; were a part of special program/trial that might affect their clinical/hematological status; were under HU therapy and those who refused consent for the study.

Informed written consent was obtained from all study participants. The research staff administered a questionnaire collecting information on socio-demographic profile, clinical manifestations and frequency of painful crisis on those who consented to participate in the study.

2.4. Detection of alpha thalassemia by PCR

Alpha thalassemia was detected by nucleic acid amplification tests. The DNA was extracted from the whole blood by a standard phenol chloroform extraction method [31]. Briefly, the DNA was extracted twice with an equal volume of phenol: chloroform:isoamyl alcohol (25:24:1) and once with an equal volume of chloroform:isoamyl alcohol (24:1). Nucleic acids were precipitated with 0.6 volumes of ice cold isopropanol for 30 min at room temperature. Following centrifugation at 12,000 rpm for 40 min, the pellet was rinsed three times with 1 ml ice-cold 70% ethanol, dried at room temperature and resuspended in 200 μl low TE buffer, pH 8 (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, pH 8.0) containing 5 μg/ml RNase A. Different primer sets were used for detection of alpha globin gene deletions that is, $\alpha-^{3.7}$ and $\alpha-^{4.2}$. The first set was for amplification of normal alpha globin gene (5′-CCCCTCGCCAAGTCCACCC-3′)/(5′- AGACCAGGAAGGGCC GGTG-3′) (1800 bp), while the second set was for $\alpha-^{3.7}$ (5′- CCCCTCGCC AAGTCCACCC-3′)/(5′- AAAGCACTCTAGGGTCCAGCG-3′) (2022 bp) and the third set for $\alpha-^{4.2}$ (5′- GGTTTACCCATGTGGTGCCTC-3′)/(5′- CCCGT TGGATCTTCTCATTTCCC-3′) (1628 bp) [32]. For QA/QC we ran a positive control and a negative control with each batch.

2.5. Statistical analysis

The data were analyzed using the statistical program Stata 11.0 (StataCorp, *Stata Statistical Software*: StataCorp LP: College Station, Texas, USA). Pearson chi square test was used to compare the socio demographic and clinical variables in both mild and severe cases. Univariate and multivariate analysis was done to identify the association and estimate the independent contribution of each variable respectively in cases and controls.

3. Results

A total of 373 SCD (HbS-only phenotype) patients fulfilling the recruitment criteria were enrolled during the study period, out of which cases were 256 and controls were 117. The gender distribution in both case and control groups were almost similar. The proportion of male patients was slightly higher in the cases (59.77%) than the controls (51.28%) and the proportion of female patients were higher in the controls (48.72%) than the cases (40.23%). But these differences were

Table 1
Sociodemographic and clinical variables.

Variables	Controls (n = 117)	Cases (n = 256)	Chi square P <
Sex			
Male	60 (51.28)	153 (59.77)	0.125
Female	57 (48.72)	103 (40.23)	
Age(yrs)			
≥ 31	19 (16.24)	31 (12.11)	
11–20	27 (23.08)	54 (21.09)	0.443
21–30	71 (60.68)	171 (66.80)	
HbF(%)			
< 15.0	21 (17.95)	67 (26.17)	
15.1–25.0	71 (60.68)	159 (62.11)	**0.025**
> 25.1	25 (21.37)	30 (11.72)	
Hb (g/dl)			
> 10.10	18 (15.38)	47 (18.36)	
< 8.0	33 (28.21)	71 (27.73)	0.776
8.1–10.9	66 (56.41)	138 (53.91)	
MCV (fL)			
< 83.0	75 (64.10)	182 (71.09)	
83.1–101	40 (34.19)	74 (28.91)	0.058
> 101.1	2 (1.71)	0	
MCH (pg)			
> 32	11 (9.40)	14 (5.47)	
< 27	66 (56.41)	156 (60.94)	0.343
27–32	40 (34.19)	86 (33.59)	
MCHC (g/dL)			
> 34.5	32 (27.35)	49 (19.14)	
< 31.5	22 (18.80)	45 (17.58)	0.154
31.5–34.5	63 (53.85)	162 (63.28)	
WBC ($*10^3$/μL)			
> 10.1	58(49.57)	123(48.05)	
< 4.0	1 (0.85)	4 (1.56)	0.840
4.0–10.0	58 (49.57)	129 (50.39)	
Platelet ($*10^3$/μL)			
> 410	19 (16.24)	61 (23.83)	
< 150	14 (11.97)	38 (14.84)	0.136
150–410	84 (71.79)	157 (61.33)	
Alpha thalassemia			
No	71 (60.68)	190 (74.22)	**0.008**
Yes	46 (39.32)	66 (25.78)	

Data are expressed in no (%). P-value determined by Pearson's chi-square (χ^2) test.
Fetal hemoglobin (HbF), hemoglobin (Hb), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean cell hemoglobin (MCH), white blood cell (WBC).
The bold data in the table indicates P value which is significant i.e p < 0.05.

statistically not significant. The age group ranged from 11 to 58 years. We excluded children aged 10 years and below since HbF fluctuates till this age. The mean age of control group was 22.05 ± 8.17 and case group was 21.89 ± 8.62; the small difference was not statistically significant.

To assess the extent of deviation in the SCD patients from the normal reference range, the hematological indices, WBC, and platelet counts were categorized in to three groups, i.e. within the reference range [33], below the reference range and above the reference range. Since reference ranges for blood hemoglobin (Hb) levels are different for males, females and children, we have categorized them into three groups, i.e., normal and mild anemia, moderate anemia and severe anemia according to WHO classification [34]. Proportion of moderate and severe anemia was very high in both case and control groups but there were no difference in the distribution of anemia in the two groups. Similarly, microcytosis and hypochromia were present in a very high proportion of subjects in both groups, but the difference between the two groups was not significant (Table 1). There was no difference in MCHC values between case and control groups. We observed leukocytosis (WBC count > 10,000/μL) and thrombocytosis (platelet count > 410,000/μL) in a significant proportion of subjects in both groups. However, the distribution of leukocytosis and thrombocytosis was not different in the two groups (Table 1).

The HbF level was also categorized into 3 groups i.e. ≤ 15%, 15.1–25%, > 25%. Low HbF (≤15%) was noticed in higher proportion in the cases (26.17%) than the control group (17.95%). In contrast, higher HbF (> 25%) was found in lower proportion in the cases (11.72%) than the control group (21.37%). However, HbF level between 15.1 and 25% was almost similar in both groups. The distribution of different HbF categories between case and control group was statistically significant (P < 0.025, $\chi 2$ test) (Table 1). We further wanted to examine if the difference of HbF distribution was age dependent. The mean HbF level at different age groups was 21.2 ± 5.62 in 11–20 years, 18.21 ± 6.11 in 21–30 years; and 20.34 ± 6.52 in 31 years or above age group in the controls and 18.46 ± 6.20 in 11–20 years; 18.61 ± 6.35 in 21–30 years; and 20.19 ± 6.96 in 31 years or above age group in cases. We did notice a significant difference in the mean HbF level in the 11–20 yrs. age group (Students '*t*' test, P < 0.001) only (Fig. 1).

Alpha thalassemia was detected in 39.32% in control group as compared to 25.78% in cases. The difference in presence of alpha thalassemia was statistically significant between both the groups (P < 0.008, $\chi 2$ test) (Table 1). We observed only $\alpha -^{3.7}$ deletion in our study population. α − 3.7 deletion was found both in heterozygous and homozygous state. In the control group, the distribution of heterozygous and homozygous state was 27.35% and 11.97% respectively. In the cases group, 18.75% subjects had heterozygous and 7.03% subjects had homozygous alpha thalassemia.

Univariate analysis of HbF, hematological indices and alpha thalassemia showed a strong reverse association between HbF level and frequency of acute pain episodes. HbF level in the group with > 25% showed strong protection against frequency of acute pain episodes (OR: 0.37, 95% CI: 0.18–0.77, P < 0.008). Presence of alpha thalassemia also showed protection (OR: 0.53, 95% CI: 0.33–0.85, P < 0.009). However, multivariate analysis showed significant protection provided only by higher HbF (OR: 0.39, 95% CI: 0.17–0.88, P < 0.025) but not by alpha thalassemia. Higher platelet counts were associated with the risk of higher frequency of acute pain episodes (OR: 0.51, 95% CI: 0.27–0.96, P < 0.037) (Table 2).

4. Discussion

HbF is the most thoroughly studied genetic modulator of sickle cell anemia with majority of studies indicating a protective role for HbF against acute pain episodes. However, there is no unanimity on whether

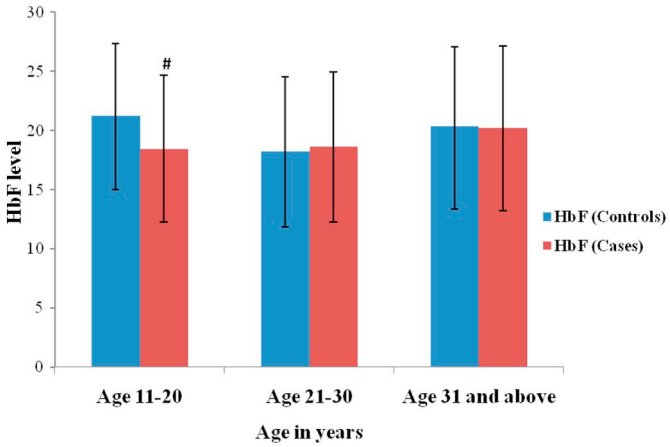

Fig. 1. HbF distribution among different age groups (P-value determined by Students '*t*' test).

Table 2
Univariate and multivariate logistic regression analysis.

Risk factor	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Sex	0.70 (0.45–1.10)	0.125	0.73 (0.46–1.18)	0.211
Age				
≥31	1.0		1.0	
11–20	1.22 (0.58–2.55)	0.587	1.34 (0.60–2.96)	0.462
21–30	1.47 (0.78–2.78)	0.229	1.60(0.80–3.19)	0.178
HbF(%)				
< 15.0	1.0		1.0	
15.1–25	0.70 (0.39–1.23)	0.219	0.73 (0.40–1.33)	0.312
> 25.1	0.37 (0.18–0.77)	**0.008**	0.39 (0.17–0.88)	**0.025**
Hb (g/dl)				
> 10.10	1.0		1.0	
< 8.0	0.82 (0.41–1.63)	0.578	0.82 (0.39–1.74)	0.614
8.1–10.9	0.80 (0.43–1.48)	0.481	0.80 (0.40–1.57)	0.518
MCH (pg)				
> 32	1.0		1.0	
< 27	1.85 (0.80–4.30)	0.149	0.68 (0.19–2.40)	0.552
27–32	1.68 (0.70–4.04)	0.240	0.86 (0.31–2.39)	0.785
MCHC (g/dL)				
> 34.5	1.0		1.0	
< 31.5	1.33 (0.67–2.62)	0.402	1.27 (0.57–2.83)	0.549
31.6–34.5	1.67 (0.98–2.85)	0.056	1.70 (0.90–3.19)	0.099
WBC($*10^3$/μL)				
> 10.1	1.0		1.0	
< 4.0	1.88 (0.20–17.25)	0.574	2.75 (0.25–29.9)	0.406
4.1–10.0	1.04 (0.67–1.62)	0.832	1.41 (0.83–2.40)	0.200
Platelet ($*10^3$/μL)				
> 410	1.0		1.0	
< 150	0.84 (0.37–1.88)	0.681	0.71 (0.27–1.81)	0.476
151–410	0.58 (0.32–1.03)	0.067	0.51 (0.27–0.96)	**0.037**
Alpha thalassemia	0.53 (0.33–0.85)	0.009	0.66 (0.40–1.10)	0.115

Data are expressed in no (%). P-value determined by Pearson's chi-square (χ^2) test.
Fetal hemoglobin (HbF), hemoglobin (Hb), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean cell hemoglobin (MCH), white blood cell (WBC).
The bold data in the table indicates P value which is significant i.e $p < 0.05$.

the inverse correlation between HbF concentration and frequency of sickle cell complications is linear or the protection is provided only beyond critical HbF level. Increased HbF has generally been found to increase survival in sickle cell anemia. In the present study, a clear inverse association was found between frequency of pain episodes and HbF concentrations. In a study from Jamaica increase in HbF from 1.0% to 4.6%–5.2% was associated with increased the packed cell volume (PCV) and mean corpuscular volume (MCV), but did not reduce the incidence of painful crises, abdominal crises and the acute chest syndrome [35]. Earlier studies also did not find any relationship between HbF and overall severity [36,37]. Powers et al. in 1984 suggested that the threshold level of HbF to prevent acute clinical events was about 20% [38]. Similar observations to the current study were found in SCD adult patients with AI haplotype suggesting that haplotype contributes to association between HbF levels and acute clinical episode [25]. HbF level is dependent on the F cell distribution in the erythrocytes and are highly heritable. Conran [39] suggested that drugs used for augmenting HbF in SCA need to be used cautiously to ensure that HbF is elevated in a pan-cellular manner. It has been reported that neither the HbF concentration nor the number of cells with detectable HbF (F-cells) can predict the severity of SCD [10]. Proportion of F-cells with protective concentrations of HbF that can prevent polymerization is presumed as the critical predictor of severe complications of SCD, F-cells with insufficient HbF concentrations may not inhibit HbS polymerization. Protective concentrations of HbF can be found in only 1–24% of cells when HbF concentration is around 20%, but rapidly rises to 70% when the total HbF concentration reaches near 30% [10]. In our study significant association was found only in cases with a HbF level of > 25%.

There are conflicting reports on the beneficial effect of alpha thalassemia on the frequency of SCD complications; while some studies have shown protection others have shown harmful effects. The coinheritance of alpha thalassemia and SCD was associated with improved hematological indices, and lower consultations rate in Cameroonian patients [40]. Kulozik et al. [41] in a study from Odisha reported higher prevalence of alpha thalassemia providing protection and increased survival of patients with sickle cell disease. However, another report has indicated higher risk for VOC events in children with SCA when associated with alpha-thalassemia [42]. A systematic review of the literature for severity predictors in children with SCA suggested that association of alpha thalassemia did not provide protection against frequency of painful crisis, but beneficial effect of increasing HbF levels was found in two thirds of the studies [43]. Another Indian study demonstrated that inverse association between HbF levels and VOC and severe anemia while presence of alpha thalassemia increased the rate of painful events and sepsis [44]. Our study revealed no such association in adjusted OR, though there was a significant association in unadjusted OR.

Hematological parameters did not differ significantly between controls and cases. However, the proportion of cases with moderate to severe anemia, low MCV and low MCH was very high in both the groups. A similar status was reported from other parts of India - moderate to severe anemia with high HbF was predominantly noticed in Central Indian SCD patient's [45,46]. Leukocytosis and thrombocytosis were present in a significant proportion of controls and cases, though there was no significant difference between the two groups. High leukocyte counts correlated with severe complications of SCD including brain infarction, hemorrhagic strokes, and acute chest syndrome [47,48]. Similarly, platelet activation is elevated in SCD patients under steady-state conditions, which is further enhanced during VOC [49,50]. Patients with elevated white blood cell counts and platelet counts were at higher risk for frequent hospital admissions [9]. We did notice association of higher platelet counts with frequent acute pain episodes. Since the present study was designed to assess association between different exposures and episodes of painful crisis, we had not measured other complications of SCD.

The current study was conducted in a population of SCD subjects where HbF concentrations were reasonably high, yet the frequency of common complications of sickling high. The most important strength of our study is that we have conducted a case control study in SCD patients depending on the frequency of acute pain episodes. Most of the earlier studies were either cross-sectional studies or a comparison of homozygous and heterozygous state. The other strength of the study was that universally accepted standard methods were used for measurement of different exposures. The main limitation of the study was the classification of cases and controls were based on history of pain episodes, thus exposing the study for recall bias, specifically in the control group. Another important limitation was that we had not measured F cell count, though we have mentioned in the discussion a protective concentration of HbF in the F cells provide protection against VOCs. Lastly, though we presume that our subjects were of AI haplotype, we have not done any confirmatory test to prove our presumption.

In conclusion, our data suggest that only higher concentration of HbF is associated with protection against frequent episodes of acute pain episodes. Thrombocytosis possibly contributes for higher frequency of pain episodes. Association of alpha thalassemia, leukocytosis and other red cell indices did not appear to influence frequency of pain episodes SCD in our cohort.

Declarations

Ethics approval and consent to participate: The study was approved by the Institutional Review Board of Asian Institute of Public health (AIPH) [ERC/No: 2014-04] [ERC/No: 2018- 29] and Veer Surendra Sai Institutional Research & Ethics Committee (VIREC) [ERC No: IEC/IRB-06/15]. Only those patients who provided written informed consent to participate in the study were included.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Bimal Jit was supported by the Department of Biotechnology, Ministry of Science and Technology, Government of India funding (Project Number: BT/Bio-CARe/07/551/2012). The funding agencies had no involvement in study design, data collection, data analysis, and data interpretation.

Authors' contributions

PD was involved in designing of the study and conceptualizing the manuscript. PKM, KD, SP, and SM were involved in recruitment of cases and controls, BPJ, PP, JRM and SS did the data collection and laboratory work. PD conducted data analysis. PKM, PP, SP, SM, KD and RKB provided insightful comments in the review of the manuscript. All authors had contributed intellectual input during the study period and contributed to redrafting the manuscript.

Acknowledgements

We would like to express our appreciation for the support from study participants, and hospital nurses for assisting in this project.

References

[1] D. Mohanty, M.B. Mukherjee, Sickle cell disease in India, Curr. Opin. Hematol. 9 (2002) 117–122.
[2] P. Purohit, S. Dehury, S. Patel, et al., Prevalence of deletional alpha thalassemia and sickle gene in a tribal dominated malaria endemic area of eastern India, ISRN Hematol. 2014 (2014) 745245.
[3] M.H. Steinberg, Pathophysiology of sickle cell disease, Baillieres Clin. Haematol. 11 (1998) 163–184.
[4] W.A. Eaton, J. Hofrichter, Hemoglobin S gelation and sickle cell disease, Blood 70 (5) (1987) 1245–1266.
[5] M.H. Steinberg, Management of sickle cell disease, N. Engl. J. Med. 340 (1999) 1021–1030.
[6] D.C. Rees, T.N. Williams, M.T. Gladwin, Sickle-cell disease, Lancet 376 (2010) 2018–2031.
[7] M.H. Steinberg, Predicting clinical severity in sickle cell anaemia, Br. J. Haematol. 129 (2005) 465–481.
[8] A. Akinbami, A. Dosunmu, A. Adediran, et al., Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA controls in Lagos, Nigeria, BMC. Res. Notes 5 (2012) 396.
[9] S.A. Curtis, N. Danda, Z. Etzion, et al., Elevated steady state WBC and platelet counts are associated with frequent emergency room use in adults with sickle cell anemia, PLoS ONE 10 (2015) e0133116.
[10] M.H. Steinberg, D.H. Chui, G.J. Dover, et al., Fetal hemoglobin in sickle cell anemia: a glass half full? Blood 123 (2014) 481–485.
[11] J. Watson, The significance of the paucity of sickle cells in newborn Negro infants, Am J Med Sci 215 (1948) 419–423.
[12] O.S. Platt, B.D. Thorington, D.J. Brambilla, et al., Pain in sickle cell disease, rates and risk factors, N. Engl. J. Med. 325 (1991) 11–16.
[13] I. Akinsheye, A. Alsultan, N. Solovieff, et al., Fetal hemoglobin in sickle cell anemia, Blood 118 (2011) 19–27.
[14] B.A. Miller, N. Olivieri, M. Salameh, et al., Molecular analysis of the high-hemoglobin-F phenotype in Saudi Arabian sickle-cell anemia, N. Engl. J. Med. 316 (1987) 244–250.
[15] N.S. Green, M.E. Fabry, L. Kaptue-Noche, et al., Senegal haplotype is associated with higher HbF than Benin and Cameroon haplotypes in African children with sickle cell anemia, Am. J. Hematol. 44 (1993) 145–146.
[16] D. Labie, J. Pagnier, C. Lapoumeroulie, et al., Common haplotype dependency of high G gamma-globin gene expression and high Hb F levels in beta-thalassemia and sickle-cell anemia patients, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 2111–2114.
[17] R.L. Nagel, S.K. Rao, O. Dunda-Belkhodja, et al., The hematologic characteristics of sickle cell anemia bearing the Bantu haplotype: the relationship between G gamma and HbF level, Blood 69 (1987) 1026–1030.
[18] C.T. Noguchi, A.N. Schechter, The intracellular polymerization of sickle hemoglobin and its relevance to sickle cell disease, Blood 58 (1981) 1057–1068.
[19] M.H. Steinberg, B.G. Forget, D.R. Higgs, et al., Disorders of Hemoglobin: Genetics, Pathophysiology, Clinical Management, Second ed., Cambridge University Press, Cambridge, United Kingdom, 2009.
[20] J. Watson, A study of sickling of young erythrocytes in sickle cell anemia, Blood 3 (1948) 468–469.
[21] D. Edoh, C. Antwi-Bosaiko, D. Amuzu, Fetal hemoglobin during infancy and in sickle cell adults, Afr. Health Sci. 6 (2006) 51-44.
[22] R.P. Perrine, M.J. Brown, J.B. Clegg, et al., Benign sickle cell disease, Lancet 2 (1972) 1163–1167.
[23] V. Brousse, S. El Hoss, N. Bouazza, et al., Prognostic factors of disease severity in infants with sickle cell anemia: a comprehensive longitudinal cohort study, Am. J. Hematol. 93 (2018) 1411–1419.
[24] S. Charache, M.L. Terrin, R.D. Moore, et al., Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia, N. Engl. J. Med. 332 (1995) 1317–1322.
[25] A. Alsultan, M.K. Alabdulaali, P.J. Griffin, et al., Sickle cell disease in Saudi Arabia: the phenotype in adults with the Arab-Indian haplotype is not benign, Br. J. Haematol. 164 (2014) 597–604.
[26] A.H. Habara, E.M. Shaikho, M.H. Steinberg, Fetal hemoglobin in sickle cell anemia: the Arab-Indian haplotype and new therapeutic agents, Am. J. Hematol. 92 (2017) 1233–1242.
[27] A. Inati, W. Taher, A.W.S. Bou, et al., β-Globin gene cluster haplotypes and HbF levels are not the only modulators of sickle cell disease in Lebanon, Eur. J. Haematol. 70 (2003) 79–83.
[28] R.B. Colah, R. Surve, P. Sawant, et al., HPLC studies in hemoglobinopathies, Indian J. Pediatr. 74 (2007) 657–662.
[29] G.R. Serjeant, K. Ghosh, J. Patel, Sickle cell disease in India: a perspective, Indian J. Med. Res. 143 (2016) 21–24.
[30] R.S. Mashon, P.M. Dash, J. Khalko, et al., Higher fetal hemoglobin concentration in patients with sickle cell disease in eastern India reduces frequency of painful crisis, Eur. J. Haematol. 83 (2009) 383–384.
[31] J.M. Old, D.R. Higgs, Gene analysis, in: D.J. Weatherall (Ed.), The Thalassemia. Methods in Hematology, 6 Churchill Levingstone, Edinburgh, 1982, pp. 74–102.
[32] S.S. Chong, C.D. Boehm, D.R. Higgs, et al., Single-tube multiplex-PCR screen for common deletional determinants of alpha thalassemia, Blood 95 (2000) 360–362.
[33] B.J. Bain, I. Bates, M.A. Laffan, Dacie and Lewis Practical Haematology, 12th edition, (2017).
[34] WHO, Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity, Vitamin and Mineral Nutrition Information System, World Health Organization, Geneva, 2011(WHO/NMH/NHD/MNM/11.1).
[35] A. Donaldson, P. Thomas, B.E. Serjeant, et al., Foetal haemoglobin in homozygous sickle cell disease: a study of patients with low HBF levels, Clin. Lab. Haematol. 23 (2001) 285–289.
[36] D.R. Powars, W.A. Schroeder, J.N. Weiss, et al., Lack of influence of fetal hemoglobin levels or erythrocyte indices on the severity of sickle cell anemia, J. Clin. Invest. 65 (1980) 732–740.
[37] C.C. Hedo, I.E. Okpala, Y.A. Aken'ova, Foetal haemoglobin levels in Nigerians with sickle cell anaemia. A revisitation, Trop. Geogr. Med. 45 (1993) 162–164.
[38] D.R. Powars, J.N. Weiss, L.S. Chan, et al., Is there a threshold level of fetal hemoglobin that ameliorates morbidity in sickle cell anemia? Blood 63 (1984) 921–926.
[39] N. Conran, High foetal haemoglobin in sickle cell disease: not so protective? EBioMedicine 2 (2015) 102–103.
[40] M.B. Rumaney, V.J.N. Bitoungui, A.A. Vorster, et al., The co-inheritance of alpha-thalassemia and sickle cell anemia is associated with better hematological indices and lower consultations rate in Cameroonian patients and could improve their survival, PLoS ONE 9 (2014) e100516.
[41] A.E. Kulozik, B.C. Kar, G.R. Serjeant, The molecular basis of alpha thalassemia in India. Its interaction with the sickle cell gene, Blood 71 (1988) 467–472.
[42] C. Renoux, P. Connes, E. Nader, et al., Alpha-thalassaemia promotes frequent vaso-occlusive crises in children with sickle cell anaemia through haemorheological changes, Pediatr. Blood Cancer 64 (2017) e26455.
[43] E.R. Meier, R.M. Fasano, P.R. Levett, A systematic review of the literature for severity predictors in children with sickle cell anemia, Blood Cells Mol. Dis. 65 (2017) 86–94.
[44] D.S. Upadhye, D.L. Jain, Y.L. Trivedi, et al., Neonatal screening and the clinical outcome in children with sickle cell disease in Central India, PLoS ONE 11 (2016) e0147081.
[45] A.V. Shrikhande, A.A. Dani, J.R. Tijare, et al., Hematological profile of sickle cell disease in central India, Indian J. Hematol. Blood Transf. 23 (2007) 92–98.
[46] S.S. Rao, J.P. Goyal, S.V. Raghunath, et al., Hematological profile of sickle cell disease from South Gujarat, India, Hematol. Rep. 4 (2012) e8.
[47] T.R. Kinney, L.A. Sleeper, W.C. Wang, et al., The cooperative study of sickle cell disease. Silent cerebral infarcts in sickle cell anemia: a risk factor analysis, Pediatrics 103 (1999) 640–645.
[48] O.S. Platt, D.J. Brambilla, W.F. Rosse, et al., Mortality in sickle cell disease. Life expectancy and risk factors for early death, N. Engl. J. Med. 330 (1994) 1639–1644.
[49] J. Villagra, S. Shiva, L.A. Hunter, et al., Platelet activation in patients with sickle disease, hemolysis associated pulmonary hypertension, and nitric oxide scavenging by cell-free hemoglobin, Blood 110 (2007) 2166–2172.
[50] T. Wun, T. Paglieroni, F. Tablin, et al., Platelet activation and platelet erythrocyte aggregates in patients with sickle cell anemia, J. Lab. Clin. Med. 129 (1997) 507–516.